MPLA-SM*

Monophosphoryl Lipid A from *S. minnesota* R595; TLR4 ligand

Catalog code: tlrl-mpla2

https://www.invivogen.com/mpla

For research use only

Version 22K24-NJ

PRODUCT INFORMATION

Contents

- 1 mg Monophosphoryl Lipid A (MPLA-SM*)

Storage

- MPLA-SM* is provided lyophilized and shipped at room temperature. Store at -20°C. Lyophilized product is stable for 1 year when properly stored.
- Upon resuspension, prepare aliquots of MPLA-SM* and store at -20°C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- Biological activity has been tested using HEK-Blue™ hTLR4 cells.
- The presence of other bacterial components (e.g. lipoproteins) is controlled using HEK-Blue™ TLR2 cells.

PRODUCT DESCRIPTION

Monophosphoryl Lipid A (MPLA-SM) is extracted from the lipopolysaccharide (LPS) of *Salmonella minnesota* Re595 (Re mutant), a rough strain of Gram-negative bacteria. The preparation is a mix of MPLA congeneric forms differing in the number of acyl chains. It has been suggested that this mix is responsible for the partial TLR4 agonist function of some preparations[1]. MPLA-SM* is a new reference in our catalog. It results from an improved process of MPLA-SM extraction. While MPLA-SM* and MPLA-SM have the same ability to activate murine TLR4, MPLA-SM* is more potent than MPLA-SM at inducing human TLR4 responses.

Note: Due to the intrinsic structural complexity of lipid A, some batch-to-batch variation may occur.

PRODUCT PROPERTIES

Specificity: TLR4 agonist
Working concentration: 3 ng -1 µg/ml (human TLR4)
10 pg -1 µg/ml (mouse TLR4)
Appearance: Clear lipidic film
Solubility: 1 mg/ml in DMSO

BACKGROUND

Monophosphoryl Lipid A (MPLA) is a natural compound extracted from the lipopolysaccharide (LPS) component of the cell wall of Gram-negative bacteria. LPS is a potent activator of the immune system. Its recognition by Toll-like receptor 4 (TLR4) leads to NF-κB and IRF activation and the production of proinflammatory cytokines and interferons, respectively[2]. Thus, LPS features many characteristics needed for an effective vaccine adjuvant. However, large uncontrolled amounts of LPS are extremely toxic and can cause devastating diseases[3].

Wild-type LPS, referred to as smooth (sLPS) comprises three covalently linked regions: a Lipid A backbone, an oligosaccharide core, and O-polysaccharide chains. Some bacteria produce a truncated LPS, without O-side chains, referred to as rough (rLPS)[4].

LPS biological activity is mediated through Lipid A recognition by TLR4 and is commensurate to Lipid A number of fatty acyl chains[3]. Hexa-acylated (6 chains) Lipid A is a highly potent TLR4 agonist, while under-acylated (4-5 chains) Lipid A induces lower or antagonistic responses[5].

Acidic extraction of Lipid A from LPS produces MPLA, which displays reduced toxicity while retaining the ability to activate TLR4[6,7]. The reduced toxicity of MPLA is attributed to the preferential triggering of the IRF pathway upon TLR4 activation, resulting in decreased induction of inflammatory cytokines[8].

METHODS

Preparation of stock suspension (1 mg/ml)

Human and mouse TLR4 activation can be achieved with 3 ng -1 µg/ml and 10 pg -1 µg/ml MPLA-SM*, respectively.

- Add 1 ml of DMSO and vortex until completely resuspended.
- Prepare aliquots of stock solution and store at -20°C. Further dilutions can be prepared using water.

Notes:

- *The suspension may appear to contain floating fine particles. Sonication may help to disperse these particles.*
- *Alternatively, MPLA-SM* can be resuspended in DMSO containing 0.2% triethylamine.*

TECHNICAL SUPPORT
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TLR4 activation using MPLA-SM*

MPLA-SM* can be used to activate TLR4 in HEK-Blue™ TLR4 cells, that were designed to study TLR4 stimulation by monitoring NF-κB activation. Stimulation of HEK-Blue™ TLR4 cells with a TLR4 agonist activates NF-κB which induces the production of SEAP (secreted embryonic alkaline phosphatase). Levels of SEAP can be easily determined using a SEAP detection medium, such as QUANTI-Blue™ Solution.
For more information visit: https://www.invivogen.com/hek-blue-tlr4

- Add 20 µl of MPLA-SM* at various concentrations in a well of a 96-well plate.
- Add 180 µl of HEK-Blue™ TLR4 cell suspension per well.
- Incubate the plate for 16-24 h at 37°C, 5% CO_2.
- Collect 20 µl of supernatant and add to a well of a 96-well plate containing 180 µl of QUANTI-Blue™ Solution.
- Incubate the plate at 37°C for 1-3 h.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.

1. Wang YQ. *et al.*,2020. MPL Adjuvant Contains Competitive Antagonists of Human TLR4. Front. Immunol. 11:577823. **2. Kuzmich, NN. *et al.*, 2017.** TLR4 Signaling Pathway Modulators as Potential Therapeutics in Inflammation and Sepsis. Vaccines (Basel) 5unol. 165(2):618-22. **3. Steimle, A. *et al.* 2016.** Structure and function: Lipid A modifications in commensals and pathogens. Int J Med Microbiol 306, 290-301. **4. Raetz CR. 1990.** Biochemistry of endotoxins. Annu. Rev. Biochem. 59, 129-70. **5. Cochet, F. & Peri, F. 2017.** The role of carbohydrates in the lipopolysaccharide (LPS)/Toll-Like Receptor 4 (TLR4) Signalling. Int J Mol Sci 18. **6. Qureshi N. *et al.*, 1982.** Purification and structural determination of nontoxic lipid A obtained from the lipopolysaccharide of *Salmonella typhimurium*. J. Biol. Chem., 257:11808-15. **7. Romero CD. *et al.*, 2011.** The Toll-Like Receptor 4 agonist monophosphoryl Lipid A augments innate host resistance to systemic bacterial infection. Infect Immun. 79: 3576–3587. **8. Mata-Haro V. *et al.*, 2007.** The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. Science. 316(5831):1628-32.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR4 Cells (human TLR4)	hkb-htlr4
HEK-Blue™ mTLR4 Cells (mouse TLR4)	hkb-mtlr4
LPS-EB Ultrapure (LPS from *E. coli 0111:B4*)	tlrl-3pelps
LPS-EK Ultrapure (LPS from *E. coli K12*)	tlrl-peklps
MPLA-SM	tlrl-mpla
MPLA-SM* VacciGrade™	vac-mpla2
MPLAs (synthetic MPLA)	tlrl-mpls
MPLAs VacciGrade™	vac-mplas
QUANTI-Blue™ Solution	rep-qbs
HEK-Blue™ Detection	hb-det2

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